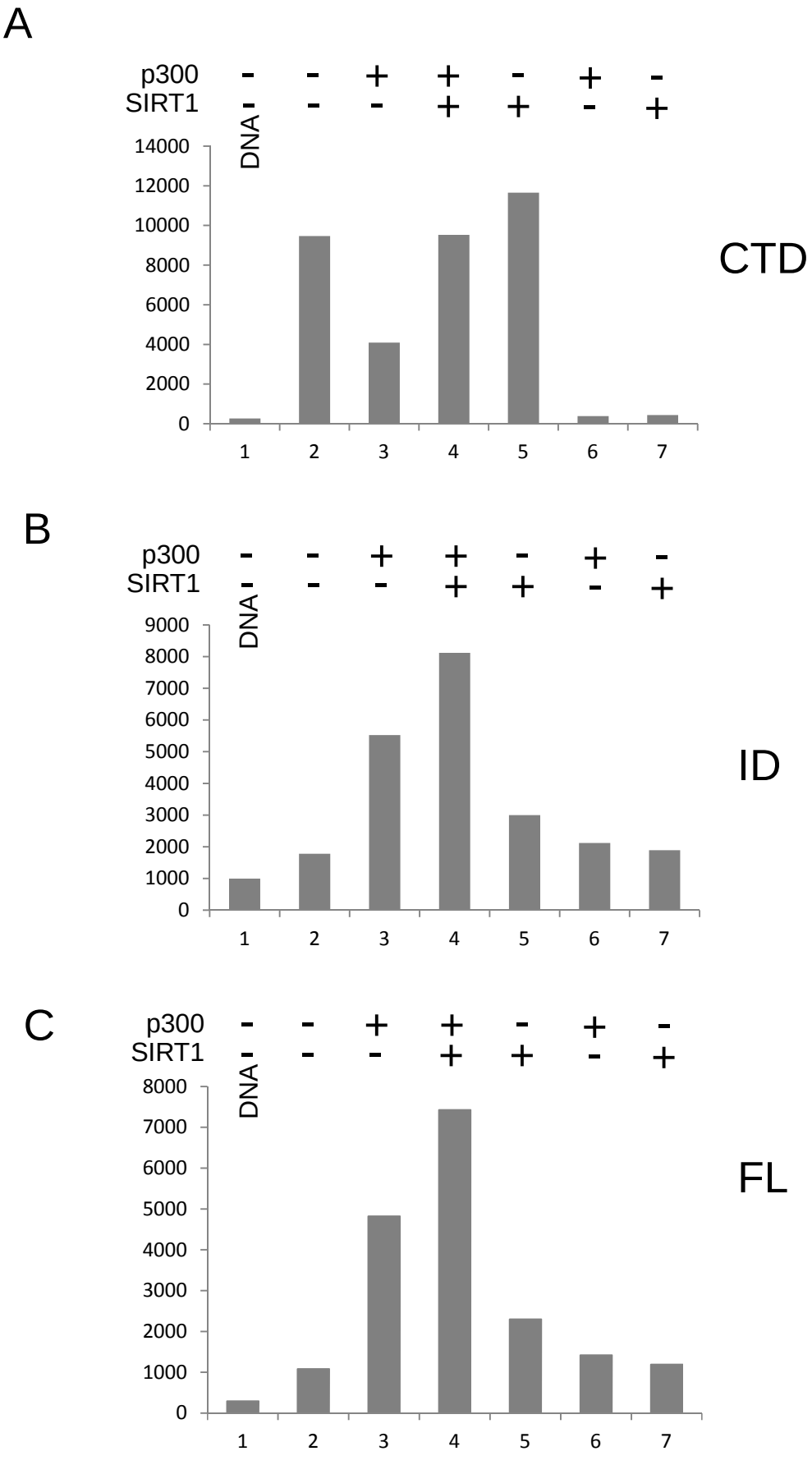
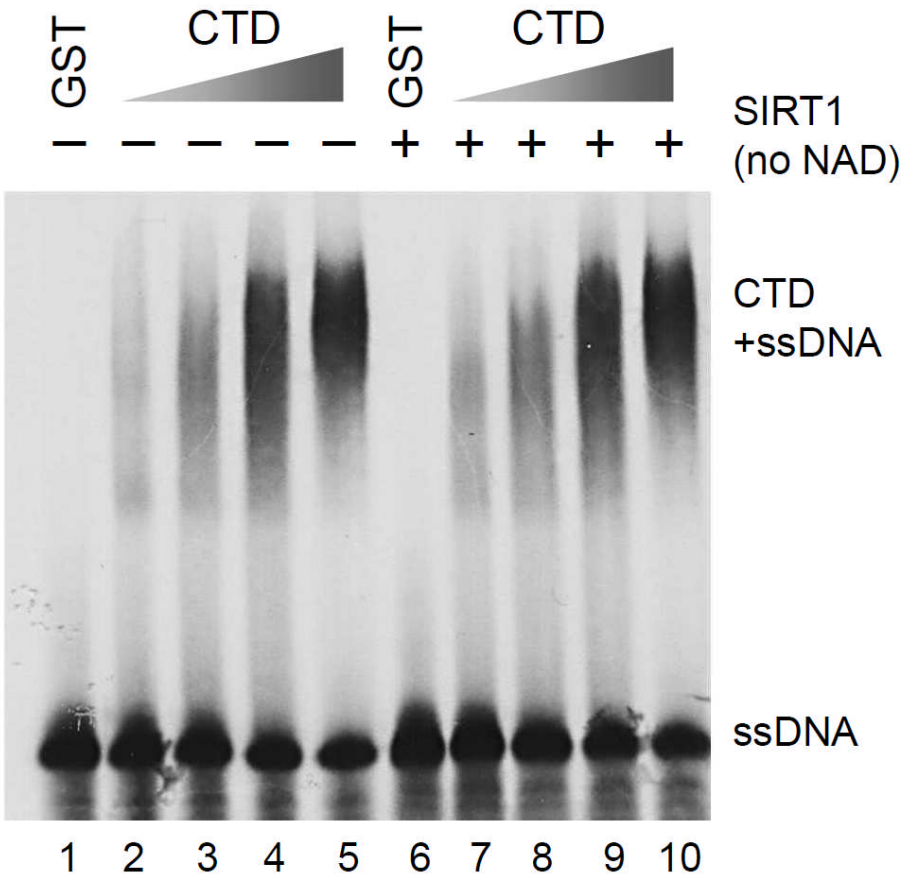
Supplementary Figure 1



Supplementary Figure 1. Acetylation and deacetylation modulate properties of Mcm10 DNA-binding. The EMSA images shown in Figure 4 were scanned and analysed using AlphaEasePC software. (A). Acetylation negates DNA-binding of CTD. Untreated CTD efficiently formed complexes with DNA (lane 2), whereas acetylated with p300 CTD did not (lane 3) leading to the ~2.5-fold drop in DNA-binding efficiency. Subsequent deacetylation by SIRT1 has restored DNA-binding by CTD (lane 4). Addition of SIRT1 (and NAD^+) to CTD did not substantially enhance its DNA-binding (lane 5, ~8% increase). Neither p300 nor SIRT1 formed complexes with ssDNA on their own (lanes 6 and 7 respectively). (B). Acetylation of the internal domain of Mcm10 (ID) enhanced its DNA-binding (lane 3 compared to 2). Subsequent deacetylation with SIRT1 did not negate it (lane 4). (**C**). Acetylation of the full-length (FL) Mcm10 stimulated DNA-complex formation (lane 3), which was further enhanced by subsequent deacetylation with SIRT1 (lane 4). Lane 1 on all gels – DNA probe without any protein.



Supplementary Figure 2. ssDNA-binding of Mcm10 CTD in the presence of SIRT1. Increased amounts of purified CTD domain of Mcm10 were incubated with radiolabeled DNA probe in the absence (lanes 2-5) or presence (lanes 7-10) of SIRT1 (without nicotinamide adenine dinucleotide (NAD^+). GST protein was used as a control for the GST-SIRT1 fusion protein was employed in the experiment (lanes 1and 10). No significant change of DNA-binding efficiency of CTD were observed (left vs. right sides of the panel). Bottom band – unbound DNA.

Supplementary Methods:

Mass spectrometry and data analysis

Peptide samples were loaded onto the LTQ-Orbitrap (Thermo Fisher Scientific, UK) using a Surveyor MS Pump and Micro AS autosampler (Thermo Fisher Scientific, UK) onto a MiChrom C18 CapTrap for desalting and then introduced into the MS via a nano-electrospray ion source consisting of a fused silica capillary column (I.D. 100 µm; O.D. 360 µm; length 20 cm; 5 µm C18 Reprosil, Nikkyo Technos Co., Japan). Separation was achieved by a dual gradient, formed of 5 - 23% Buffer B for 65 min, followed by 23% - 40% Buffer B for 30 min, and a step gradient to 60% Buffer B for 5 min (Buffer A = 0.1% formic acid; Buffer B = 100% acetonitrile (ACN), 0.1% formic acid). The pump rate was reduced via a splitter to 0.9 µl/min. Measurements were performed in the positive ion mode. The full scan precursor MS spectra (450 – 1600 m/z) were acquired in the Orbitrap analyzer with a resolution of r = 60,000. This was followed by data dependent MS/MS fragmentation of the most intense ion from the survey scan using collision induced dissociation (CID) in the linear ion trap (normalized collision energy 35%, activation Q 0.25; electrospray voltage 1.4 kV; capillary temperature 200°C; isolation width 2.00). This MS/MS scan event was repeated for the top 6 peaks in the MS survey scan. Target ions already selected for MS/MS were dynamically excluded for 40 sec. Singly charged ions were excluded from MS/MS analysis.

XCalibur software version 2.0.7 (Thermo Fisher Scientific, UK) was used for data acquisition. Raw MS files were analysed by the Mascot search engine (version 2.2, Matrix Science, London, UK) and searched against a concatenated International Protein Index (IPI) human protein database (version 3.54; containing 151376 entries and commonly observed contaminants such as porcine trypsin and some human keratins). Mascot search analysis parameters included: trypsin enzyme specificity, allowance for 2 missed cleavages, peptide

mass tolerance of 20 ppm for precursor ions and fragment mass tolerance of 0.8 Da. Methionine oxidation and lysine acetylation were selected as variable modifications and cysteine carbamidomethylation was selected as a fixed modification.

Supplementary Table 1: Identification of Mcm10 lysine (K) acetylation sites

(values for top-scoring peptide spectral matches shown below)

Sample Name	Peptide sequence	Peptide ppm	No. missed cleavages	Peptide mascot score	Peptide expect score	Position in sequence	Deacetylated by SIRT1 (Yes/No?)
ID_acetylated							
	R.VSSTEMNKK.M + Acetyl (K)	2.52	1	62	0.00013	K267	no
	K.VTPQSVNSGKTFSIWK.L + Acetyl (K)	**0.89**	**1**	**62**	**0.00018**	**K312**	**yes**
	K.TFSIWKLNDLR.D + Acetyl (K)	9.38	1	76	4.9e-06	K318	no
	K.VLIMGEALDLGTCKAK.K + Acetyl (K)	**9.53**	**1**	**62**	**0.00015**	**K390**	**yes**
ID_deacetylated							
	R.VSSTEMNKK.M + Acetyl (K)	9.18	1	36	0.05	K267	
	K.TFSIWKLNDLR.D + Acetyl (K)	5.54	1	76	5.2e-06	K318	
CT_acetylated							
	K.LSALAEAKK.L + Acetyl (K)	-2.00	1	78	3.8e-06	K657	no
	K.KLAAITKLR.A + Acetyl (K)	-1.13	2	59	3.2e-05	K664	no
	K.TNPNSIKK.K + Acetyl (K)	-1.57	1	52	0.002	K681	no
	K.TNPNSIKKK.Q + 2 Acetyl (K)	**-2.35**	**2**	**44**	**0.0088**	**K681 & K682**	**yes**
	K.KQKDPQDILEVK.E + Acetyl (K)	**1.40**	**2**	**57**	**0.00038**	**K683**	**yes**
	K.AKSKHTGILK.E + 2 Acetyl (K)	**-0.74**	**2**	**60**	**6.3e-05**	**K737 & K739**	**yes**
	K.SKHTGILKEAEAEMQER.Y + Acetyl (K)	**-3.46**	**2**	**56**	**0.00061**	**K745**	**yes**
	R.YFEPLVKK.E + Acetyl (K)	**-5.56**	**1**	**39**	**0.018**	**K761**	**yes**
	K.KEQMEEKMR.N + Acetyl (K)	**-6.44**	**2**	**41**	**0.0091**	**K768**	**yes**
	R.VVTCKTCAYTHFK.L + Acetyl (K)	9.37	1	46	0.0052	K783	no
	R.DGMLKEKTGPK.I + 2 Acetyl (K)	**7.32**	**2**	**60**	**0.00031**	**K847 & K849**	**yes**
	K.TGPKIGGETLLPR.G + Acetyl (K)	-4.99	1	83	7e-07	K853	no
	R.GEEHAKFLNSLK.- + 2 Acetyl (K)	**1.55**	**1**	**64**	**8.6e-05**	**K868 & K874**	**yes**
	K.KQKDPQDILEVK.E + 2 Acetyl (K)	**-0.60**	**2**	**49**	**0.0025**	**K683 & K685**	**yes**
	K.GQVLTKTNPNSIKK.K + 2 Acetyl (K)	**-2.99**	**2**	**(76)**	**4.1e-06**	**K674 & K682**	**yes**
	K.GQVLTKTNPNSIKK.K + 2 Acetyl (K)	-2.58	2	109	2.2e-09	K674 & K681	no
	R.AKGQVLTKTNPNSIK.K + 2 Acetyl (K)	-3.77	2	120	1.8e-10	K668 & K674	no
CT_deacetylated							
	K.LSALAEAKK.L + Acetyl (K)	-0.56	1	71	2e-05	K657	
	K.KLAAITKLR.A + Acetyl (K)	-1.47	2	55	7.2e-05	K664	
	K.TNPNSIKK.K + Acetyl (K)	-2.21	1	42	0.02	K681	
	R.VVTCKTCAYTHFK.L + Acetyl (K)	-1.96	1	44	0.0073	K783	
	K.TGPKIGGETLLPR.G + Acetyl (K)	-0.31	1	62	9.3e-05	K853	
	K.GQVLTKTNPNSIKK.K + 2 Acetyl (K)	-3.33	2	85	5.1e-07	K674 & K681	
	R.AKGQVLTKTNPNSIK.K + 2 Acetyl (K)	-2.18	2	64	6e-05	K668 & K674	